

# Antibody Internalization Detection

## Standard Operating Procedure (SOP)

Cat. No.	Description
AME100003	DiTag™ MMAE IgG labeling reagent

### 1. AME100003 incubation with antibodies

- 1.1 Dissolve lyophilized AME100003 powder with ddH<sub>2</sub>O according to COA.
- 1.2 Prepare a 4X working solution of the tested antibody in cell culture medium. To begin, a concentration of 1μg/ml is recommended for testing purposes, thus resulting in a 4μg/ml working solution.
- 1.3 Prepare a 4X working solution of AME100003 in cell culture medium. The tested antibody and AME100003 were mixed at a mass ratio of 9:1 (molar ratio of 2:1).
- 1.4 Mix equal volumes of the 4X working solutions of the tested antibody and AME100003. Incubate the mixture at 37 °C for 1 hour to obtain the Ab-AME100003 complex at a 2X working solution concentration.

### 2. Incubation of Ab-AME100003 Complex with Cells

- 2.1 Collect and wash cells, adjusting the cell concentration using complete culture medium based on the growth rate of cells. A cell concentration of 1×10<sup>5</sup> cells/mL is recommended. Add 50μL of the cell suspension into each well of a 96 well plate.
- 2.2 Add 50μL of the Ab-AME100003 complex (2X working solution) to each well. For adherent cells, add the complex after cell adhesion. Incubate the plate in a 5% CO<sub>2</sub> incubator at 37°C.

### 3. Detection of Killing Effect

After incubating for 2-3 days, assess the killing effect of the tested antibody using the CCK8 or CTG method following the manufacturer's protocols.

